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"MICROSTIMULATION OF THE LUMBOSACRAL SPINAL CORD"

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THIS QPR IS BEING SENT TO YOU BEFORE IT HAS BEEN REVIEWED BY THE STAFF OF THE NEURAL PROSTHESIS PROGRAM.

Abstract

In this report we discuss the results from two chronic and two acute experiments. The acute experiments were designed to monitor pressure responses within the bladder and entire length of the urethra as the spinal cord was stimulated to elicit an increase in bladder pressure. Baseline measurements were then compared to those taken after transection of the spinal cord at the $T_{9/10}$ level. Results indicated that bladder pressure increases could still be elicited by the stimulation but that their magnitude was significantly reduced. In addition, while this stimulation did not affect the intraurethral pressure at the level of the external urethral sphincter before the transection, afterward the stimulation co-activated the bladder and EUS.

In one chronic study, two 3-electrode linear arrays were implanted in the spinal cord for 21 days and then two electrodes were pulsed with our standard chronic stimulation paradigm (12 hrs on each of 2 successive days). This produced enhanced neuronal excitability near one electrode and depression of neuronal excitability near the other. Using this data together with past chronic stimulation experiments demonstrated that the chronic stimulation has a significant cumulative effect.

In this chronic experiment, autopsy revealed considerable variability in the placement of the electrodes. One of the six electrodes in this animal was associated with a large inflammatory lesion consisting of some lymphocytes, mononuclear cells, and a few neutrophils. Two electrodes were encapsulated within the dorsal pial meninges; two other electrodes penetrated through the spinal cord to the ventral pial surface. Tissue changes were similar to that documented in previous reports including gliosis, vascular hypertrophy and hyperplasia within the vicinity of the electrode tracks, and perivascular cuffing of some vessels associated with inflammatory foci. The overall impression of the smaller (shaft) 24 μm electrodes used in the chronic animal was that less tissue injury was observed using the smaller electrodes compared to the larger 50 μm electrodes used in previous experiments.

A second chronic animal has been implanted with two similar but smaller arrays and will be reported in the next quarter.

Introduction

The overall goals of this contract are to develop a method of chronic microstimulation of the sacral cord of the cat to effect micturition, and to evaluate the effects of the electrical stimulation on neural and surrounding tissues. In this report we discuss the results from one chronic experiment in which four microelectrodes implanted in the spinal cord for 21 days were stimulated continuously for 12 hrs on each of 2 successive days. Results demonstrated that this prolonged stimulation depressed neuronal excitability near one electrode but enhanced excitability near a second electrode. Neuronal excitability results to date are summarized for all chronically pulsed electrodes. A correlation is described between the electrophysiologic and histologic data.

Two acute experiments were conducted aimed at exploring the response of the bladder and urethra to microstimulation of the spinal cord before and after spinal cord transection. Results showed that transection can quickly alter the normal coordination between bladder and sphincter.

Methods

Chronic experiments. Adult male cats were anesthetized with 50% nitrous oxide and 1-2% Halothane and the spinal cord exposed as described previously. The S_2 region of the spinal cord was localized by stimulation of the dermatome it serves while recording the dorsal cord potential supradurally, as described previously. Four activated iridium microelectrodes (24-50 μm dia., 2.8 mm long, 2000 μm^2 exposed stimulating surface) were implanted manually at approximately the dorsal midline of the spinal cord and angled laterally at about 10 degrees. No matrix was used to support the electrodes. The electrodes were pulsed individually and the bladder lumen pressure was monitored. The electrodes were advanced into the cord until good elevation of bladder pressure was produced by the stimulation. The dura was then closed over the electrodes and the effect of the stimulation measured again. A silastic pad (to which the stimulating electrode leads had been glued) was sutured to the dura to reduce traction on the electrodes. The ground (indifferent) electrode was sutured in place over the microelectrodes. A small hole was made in the dura and a recording electrode inserted so as to lie approximately 3 cm caudal to the stimulating electrodes. A suture was used to secure the recording electrode to

the dura. A reference electrode was sutured to the muscle 2 cm above the dura. The wound was flushed with antibacterial solution and the muscle and skin were closed in layers. Subsequent recordings were made with the animal anesthetized with Pentothal (i.v., as needed) or Nembutal (i.v., as needed). A sterile catheter and sterile saline were used during recording of the bladder lumenal pressure.

Acute experiments. Two adult male cats were anesthetized with 50% nitrous oxide and 1-2% Halothane. The spinal cord was exposed using a standard dorsal laminectomy and the rostral extent of the S_2 region was localized in the manner described above. The spinal cord was covered with light mineral oil to prevent drying. The stimulating electrodes were inserted into the S_2 region using a standard stereotaxic apparatus.

A small pressure transducer (transducer ~1.3 mm dia, catheter ~0.8 mm dia, Millar Instruments) was passed along the length of the urethra and then slowly withdrawn at a constant rate using an electric motor. The frequency response of this pressure transducer is flat to 10 KHz. A potentiometer coupled to the shaft of the motor allowed measurement of the distance the catheter was withdrawn as a function of time. Both the pressure and position signals were digitally stored on tape (18.5 KHz sampling rate per channel). These signals were then digitized off-line at 10 Hz per channel for further analysis.

Histology. Within 20 minutes of the end of an experiment the animal was anesthetized with Nembutal and perfused through the aorta with saline followed by 4 L of 1/2 strength Karnovsky's fixative (2% paraformaldehyde and 2.5% glutaraldehyde) in 0.1 M sodium cacodylate buffer, pH 7.4. The animal was maintained in a refrigerated cold room until necroscopic examinations were performed, usually 24-48 hours after perfusion. With the electrodes *in situ*, the complete cord and spinal roots were dissected out to precisely localize the microelectrodes. Two-mm-thick transverse sections containing the electrode tracks were dissected, processed and embedded in epoxy resin. One-μm thick sections were cut serially through the blocks and examined using light microscopy.

Results

Two acute and two chronic experiments have been initiated or completed during this quarter.

The two acute experiments (SP-83 and SP-84) were aimed at evaluating the response to stimulation of the S_2 spinal cord before and after transection of the cord at the $T_{9/10}$ level. Results indicated that before the transection, the stimulation elicited significant ($p < 0.05$ for all comparisons) increases in mean bladder pressure above baseline similar to what we have reported in the past. This stimulation, however, did not cause a significant change in urethra pressure at the level of the EUS or a significant change in penile urethra pressure. Figure 1 shows the intraurethral pressure profiles 1 minute before and then 30 and 60 minutes after transection. The arrows indicate the points at which the various pressure measurements were taken.

After the cord was transected, there was no significant change in baseline bladder pressure and urethra pressure at level of EUS. Interestingly, there was a significant elevation of baseline penile urethra pressure in 1 of 2 cats. Using the same stimulation parameters as those used before the transection, we again found that the stimulation was able to evoke a significant increase in the mean bladder pressure above baseline. However, the magnitude of the elicited pressure change was significantly less than that generated prior to the transection. Following cord transection, there was a significant stimulation-evoked increase in urethra pressure at level of EUS in 1 of 2 cats, perhaps a sign of developing dyssynergia. There was no significant stimulation-evoked change in penile urethra pressure.

In the chronic experiments (SP-82 and SP-85), two, three electrode arrays were implanted. Each array consisted of three 34 μm diameter, activated iridium electrodes with straight 25 μm diameter platinum leads. In SP-82, the matrix holding the three electrodes was formed from silastic. Two problems were noted at the time of implant. First, the matrix was too large (~0.8 mm wide) to allow the two arrays to be inserted at the same rostro-caudal level and, instead, they were aligned along the midline of the cord, one rostral to the other, and angled to opposite sides of the cord. Second, the silastic backing was somewhat flexible and, when grasped with forceps, tended to force the electrodes out of alignment. As an alternative, in SP-85, the matrix was formed from a solid epoxy. This matrix was much smaller (~0.02 mm wide) than that used in SP-82 and allowed the arrays to be inserted at the same rostro-caudal level. The arrays were inserted through a single 1.5 cm dural incision.

In SP-82, all 6 electrodes were electrically viable immediately after implantation. However, 11 days following implantation, electrodes 5 and 6 each had a low impedance

indicating damage to the insulation of the electrodes or leads. The other 4 electrodes maintained relatively stable impedance over the three week implantation period. Two of the electrodes showed an evoked response in the ventral roots with a threshold below 25 μV for electrode 1 and below 15 μV for electrode 3, suggesting appropriate placement within the spinal cord.

Figure 2 shows the recruitment curves for electrode 1, at the start and end of each 12 hour stimulation session. It can be seen that after 12 hours of stimulation, the activation threshold is unchanged. However, at higher stimulus intensities, the amplitude of the evoked compound action potential is significantly increased. The implication is that neurons near the electrode stimulating surface are relatively unchanged but that more distant neurons are now easier to recruit. This enhanced neuronal excitability remains after 12 hours of rest, although it is noteworthy that the threshold is also lowered, suggesting enhanced excitability of neurons near the electrode stimulating surface. The second 12 hour period of continuous stimulation did not change the recruitment curve significantly.

The recruitment curves for electrode 3 from the same experiment are shown in Figure 3. The response of the neural tissue near the stimulating surface of this electrode is more representative of our experience employing this chronic stimulation paradigm. The evoked potential has a threshold of approximately 17 μA. After 12 hours of continuous stimulation, the threshold has shifted slightly to about 22 μA. After 12 hours of rest, the recruitment curve is nearly identical. However, after the second 12 hour period of stimulation, the threshold has shifted significantly (~33 μA) and the amplitude of the evoked response is clearly decreased across the entire range of stimulus inputs. This suggests a cumulative effect in depression of neuronal excitability.

In this chronic experiment, all six electrodes were identified at autopsy. The electrodes were grouped in arrays of three as expected. The spinal cord sections demonstrated minimal compression produced by the silastic electrode matrix (Fig. 4). Electrodes #1 - #3 were located within the left hemisphere. A large inflammatory focus was seen along the entire length of electrode #1 within the capsule surrounding the electrode and also associated with the tip. This electrode was located within the left ventral gray matter (Fig. 5a, b). The inflammatory focus was composed primarily of a mixture of lymphocytes, mononuclear cells, and macrophages as well as debris-laden phagocytic cells and few neutrophils (Fig. 5c). Electrode #3 was located within the left dorsal pial surface, the tip of which was ensheathed within a mixture of fibroblasts

and multinucleated giant cells (Fig. 6). The tip of this electrode was not associated with an inflammatory focus. Electrode #2 (and its tip), however, was also positioned within the dorsal pial surface. This electrode tip was associated with inflammatory cells that included small lymphocytes (Fig. 7), having a thicker capsule but similar cellular content compared to electrode #1. The tips of electrodes #4 and #5 penetrated the ventral pial surface on the right hemisphere (Fig. 8), while the tip of electrode #6 was observed within the medio-lateral white matter without an inflammatory focus. Besides inflammatory cells, other tissue changes observed in this animal included vascular hypertrophy and hyperplasia, gliosis and myelin changes including Wallerian degeneration, remyelinated fibers, as well as debris-laden macrophages. That is, all the features that have been described in this model in previous quarterly reports. In this animal, little neuronal changes were noted.

In SP-85, two electrodes were broken during implantation. These electrodes had become dislodged from their protective silicon "boots" at some point during the sterilization process and subsequent handling caused them to bend significantly. An attempt to straighten the shafts during surgery led to work hardening and then fracture near where the electrodes emerged from the epoxy matrix. The other 4 electrodes were implanted successfully and had appropriate impedances. This animal will undergo our chronic stimulation regime and the results will be reported in the next report.

Table 1 summarizes the physiologic results of our chronic stimulation experiments to date. In all cases, the stimulation was 80 μA, 400 μs/ph, 20 Hz, continuous (32 nC/ph, 1,600 $\mu C/cm^2$). The level of neuronal excitability enhancement or depression has been assigned to one of five levels for ease of comparison. The table indicates a comparison to the baseline excitability of that seen at the end of the first 12 hours of stimulation (End 1), the start of the second 12 hour period (Start 2), and the end of the second 12 hour stimulation period (End 2). The start of the second period is the response after 12 hours of rest.

The results indicate that there does appear to be depression of neuronal excitability and that the effect is cumulative. In only 1 of 14 electrodes did 12 hours of continuous stimulation cause significant depression of neuronal excitability. After the second stimulation period, 8 of 14 showed either moderate or significant depression of excitability. A χ^2 test was performed by grouping together the cases that produced either no change, slightly enhanced, and enhanced excitability, considered "not physiologically damaged" and grouping together the cases that

produced either slightly depressed or depressed excitability, considered "physiologically damaged." This test indicated that the second period of chronic stimulation significantly ($p < 0.02$) increased the rate of depressed neuronal excitability.

Discussion

The acute experiments in which the spinal cord was transected at the $T_{9/10}$ level indicated that spinal shock diminished, but did not eliminate, the ability to activate the bladder using intramedullary stimulation at the S_2 level. Before the transection, stimulation at the site producing a bladder pressure increase did not significantly affect intraurethral pressure at the level of the EUS. After transection, stimulation at the same site did produce co-activation of the EUS. This confirms that spinal cord transection in the cat leads to relatively fast changes in bladder/sphincter coordination. In future experiments we will test whether the EUS pressure decreases noted during stimulation of certain regions of the cord remain effective following such a transection. A positive outcome would indicate that such stimulation-induced intraurethral pressure relaxation may be an effective strategy to increase the completeness of bladder emptying.

The results from these and previous chronic experiments indicate that the spinal cord can withstand significant periods of stimulation at a functional level (i.e., 12 hours at 80 μA, 400 μs/ph, 20 Hz, continuous) without displaying signs of depressed neuronal excitability. However, it is also clear that repeated stimulation periods may have a cumulative effect. Further, a period of 12 hours of rest does not appear to be sufficient to prevent such an effect.

Histologic results during this quarter together with the observations from previous experiments emphasize that, in spite of the occasional electrode movement, smaller electrodes seemingly produce fewer tissue changes than do the larger electrodes. Thus, application of smaller electrodes appears to be a reasonable strategy for future research applications in this model.

The appearance of leukocytes within tissue is an indication that an inflammatory response has occurred. The question concerning what stimulates the influx of the leukocytes in our cat model is both intriguing and presently unclear. Leukocytes were observed along the entire path of pulsed electrode #1 and not exclusively at the tip. Similar cells were also observed near the

tip of unpulsed electrode #2. Together, this suggests that the cause of the inflammatory reaction in this animal is not related to the chronic stimulation.

Future Work

In the next quarter we plan to continue to test our new chronic design featuring two, 3-microelectrode arrays arranged longitudinally. We also plan to conduct acute studies to determine the effect of spinal cord transection on the stimulation-induced decrease in intraurethral pressure at the level of the EUS.

We will continue to examine the role of inflammatory cell infiltrates to look for possible correlations between leukocyte presence at the tips of pulsed electrodes. To this end, we are continuing ongoing studies applying specific monoclonal and polyclonal anti-cat CD antigens (as they become available) to identify specific subsets of leukocytes using immunocyto-chemical approaches.

Effects of Acute Spinal Transection

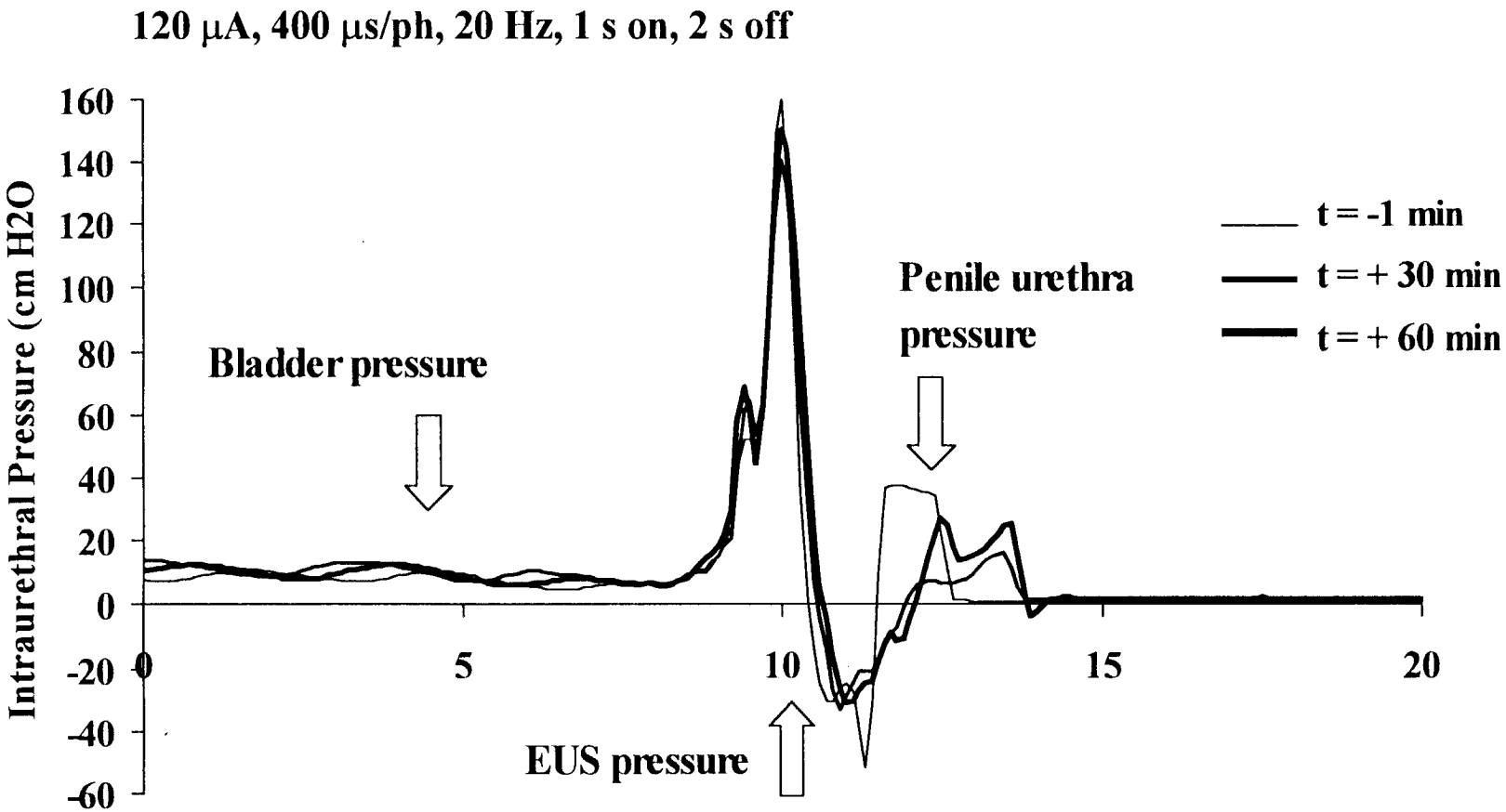

Fig. 1

SP-82 Electrode 1

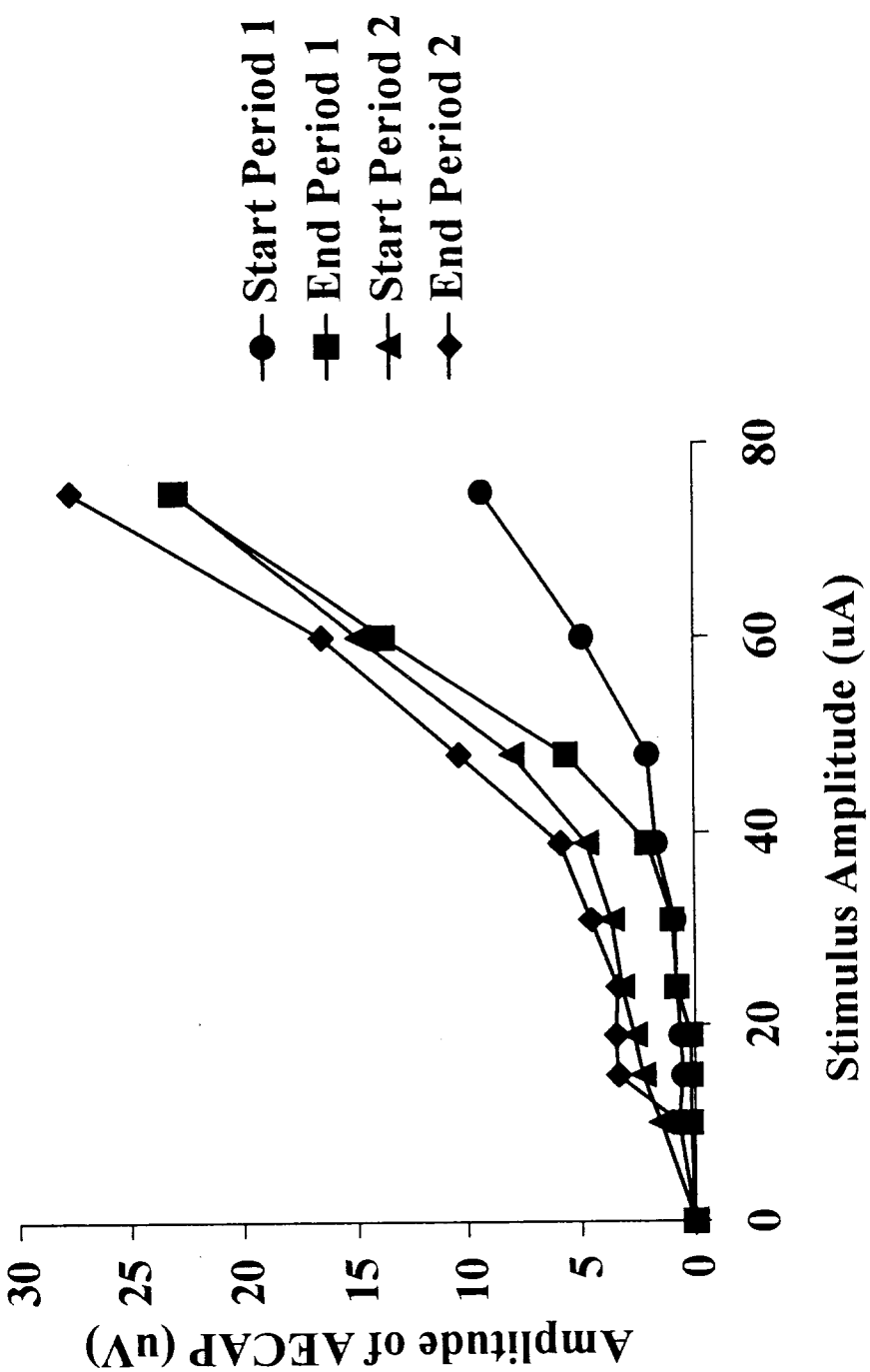

Fig. 2

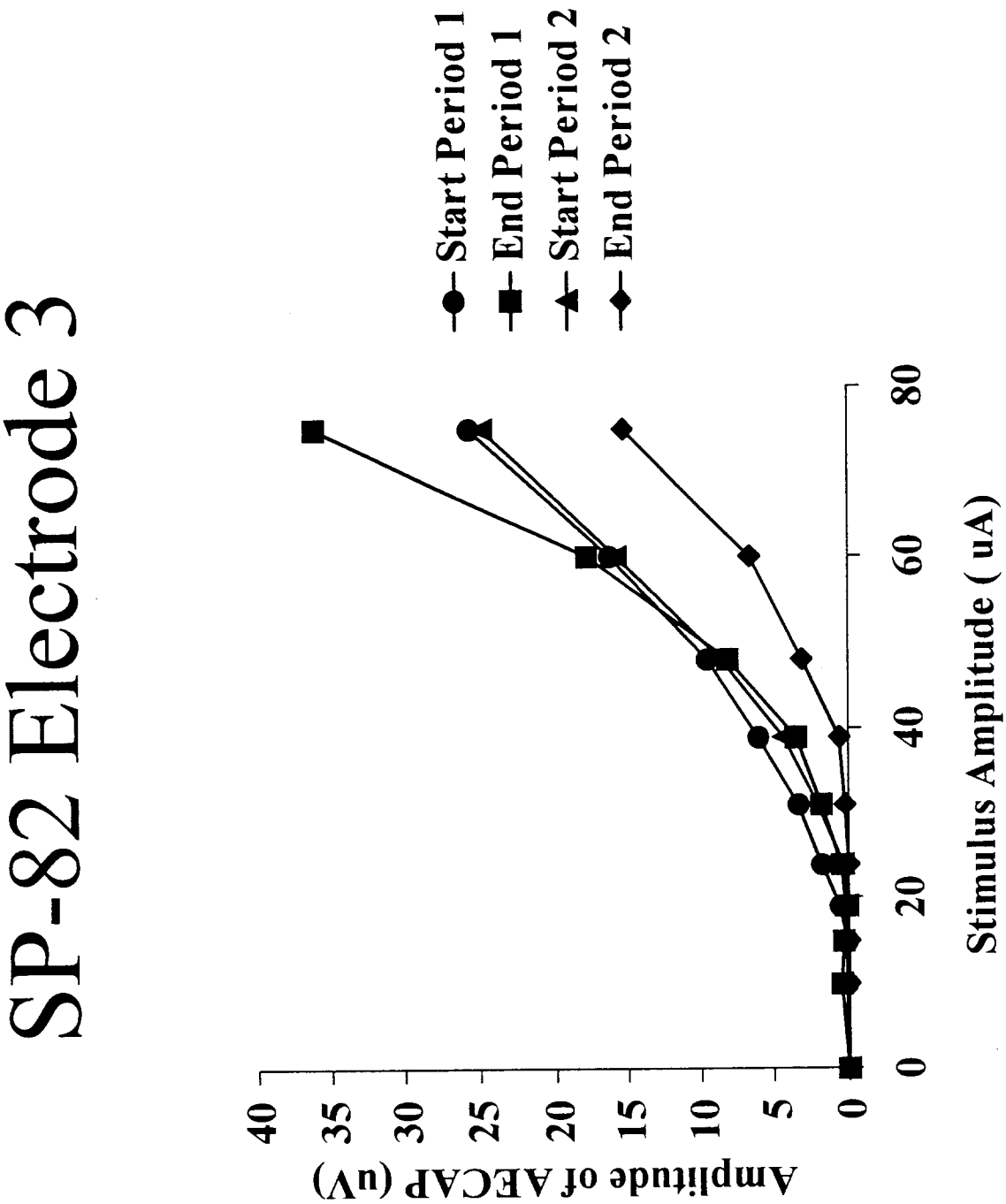
SP-82 Electrode 3
Start Period 1
End Period 1
Start Period 2
End Period 2
Amplitude of AECAP (uV)
0
5
10
15
20
25
30
35
40
0
20
40
60
80
Stimulus Amplitude (uA)

Fig. 3

Summary Chronic Stimulation

Prep	Electrode	End 1	Start 2	End 2
SP-58	2	○	○	○
	4	○	○	○
SP-61	1	○	○	◍
	3	○	○	●
SP-65	2		◍	◍
	4		◍	◍
SP-70	1	○	○	●
	2	○	○	●
SP-71	3	○		◍
	4	◍		◍
SP-73	1	●	●	●
SP-77	4		◍	◍
SP-82	1	◍	◍	◍
	3	◍	○	●

Neuronal Excitability

- ◍ **Enhanced**
- ◍ **Slightly enhanced**
- ○ **No change**
- ◍ **Slightly depressed**
- ● **Depressed**

TABLE 1

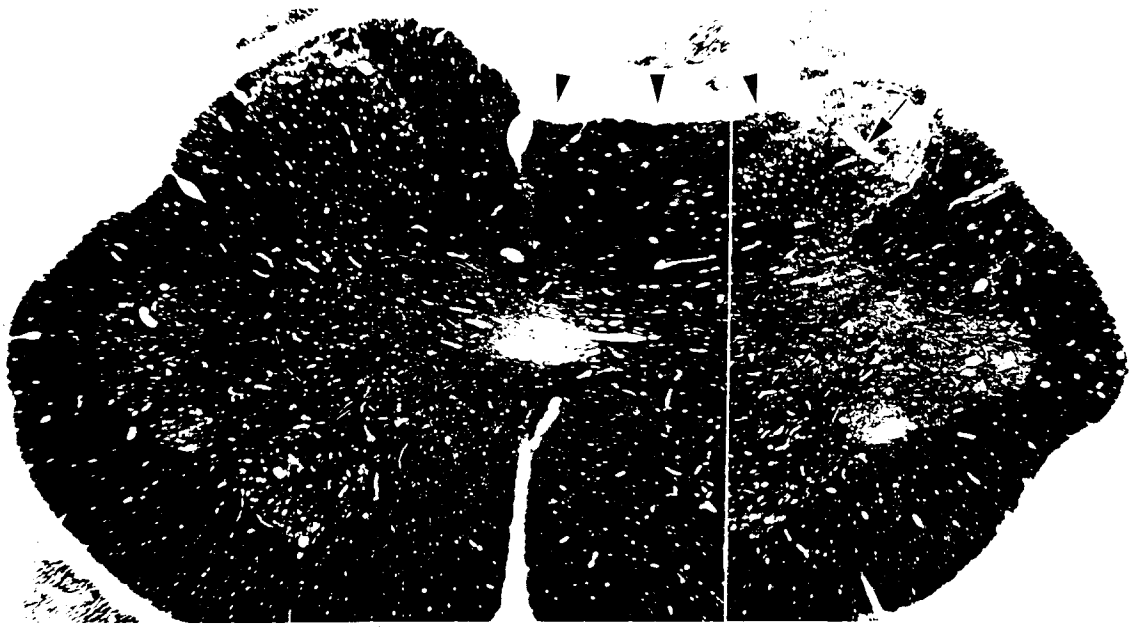

Fig. 4. SP-82. Note the compression of the dorsal surface of the spinal cord (arrowheads), produced by the connecting bar of the three electrode array. A portion of the electrode track is also shown (arrow). Bar = 500μm.

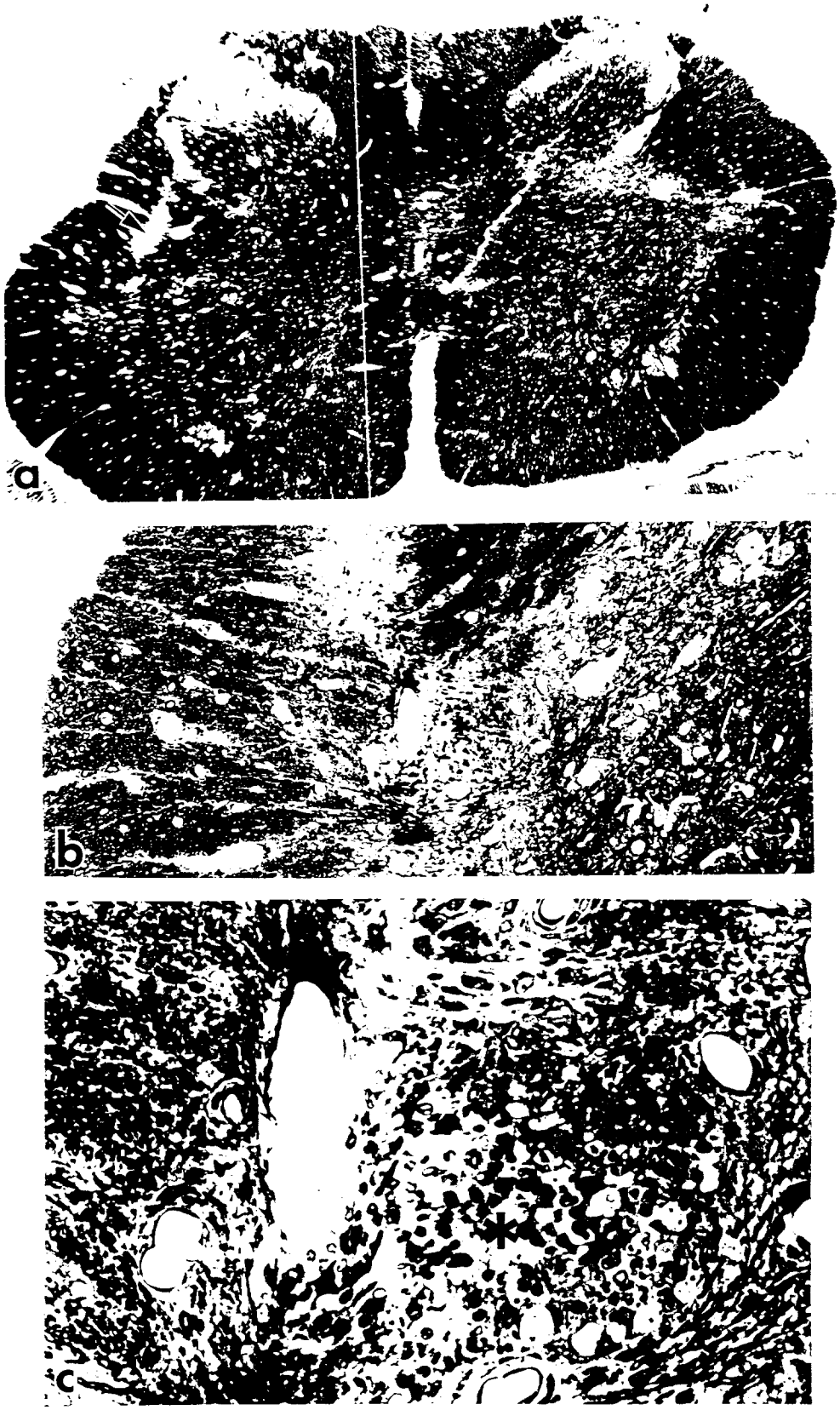

Fig. 5a-c. a. SP-82, the track made by pulsed electrode #1 is shown in the left hemisphere (arrow). b, c. A large leukocytic infiltrate was observed along the entire length of the electrode track and also near the tip of this electrode (*). The leukocytic infiltrate was composed of mononuclear cells, machrophages and few neutrophils. Bars: a = 500μm; b = 100μm; c= 25μm.

Fig. 6. SP-82, pulsed electrode #3 is shown within the dorsal meningeal surface. A typical foreign body capsule is seen encasing the electrode and its tip without an inflammatory cell infiltrate. Bar = 500μm.

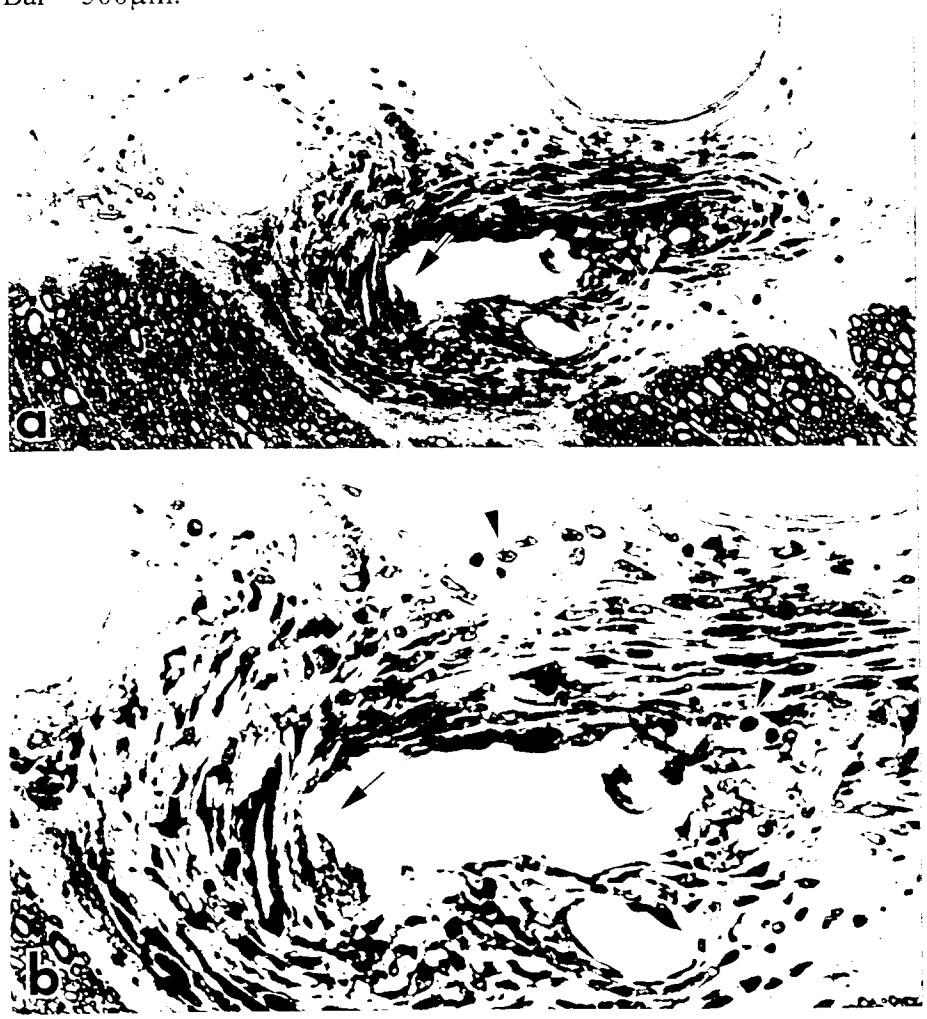

Fig.7a,b. SP-82, unpulsed electrode #2 is shown in a and b demonstrating a thicker capsule (compared to electrode #3, Fig. 6) surrounding the electrode and its tip (arrows). Note the scattered leukocytes surrounding the capsule and near the electrode tip (arrowheads). Bars: a = 50μm; b = 100μm.

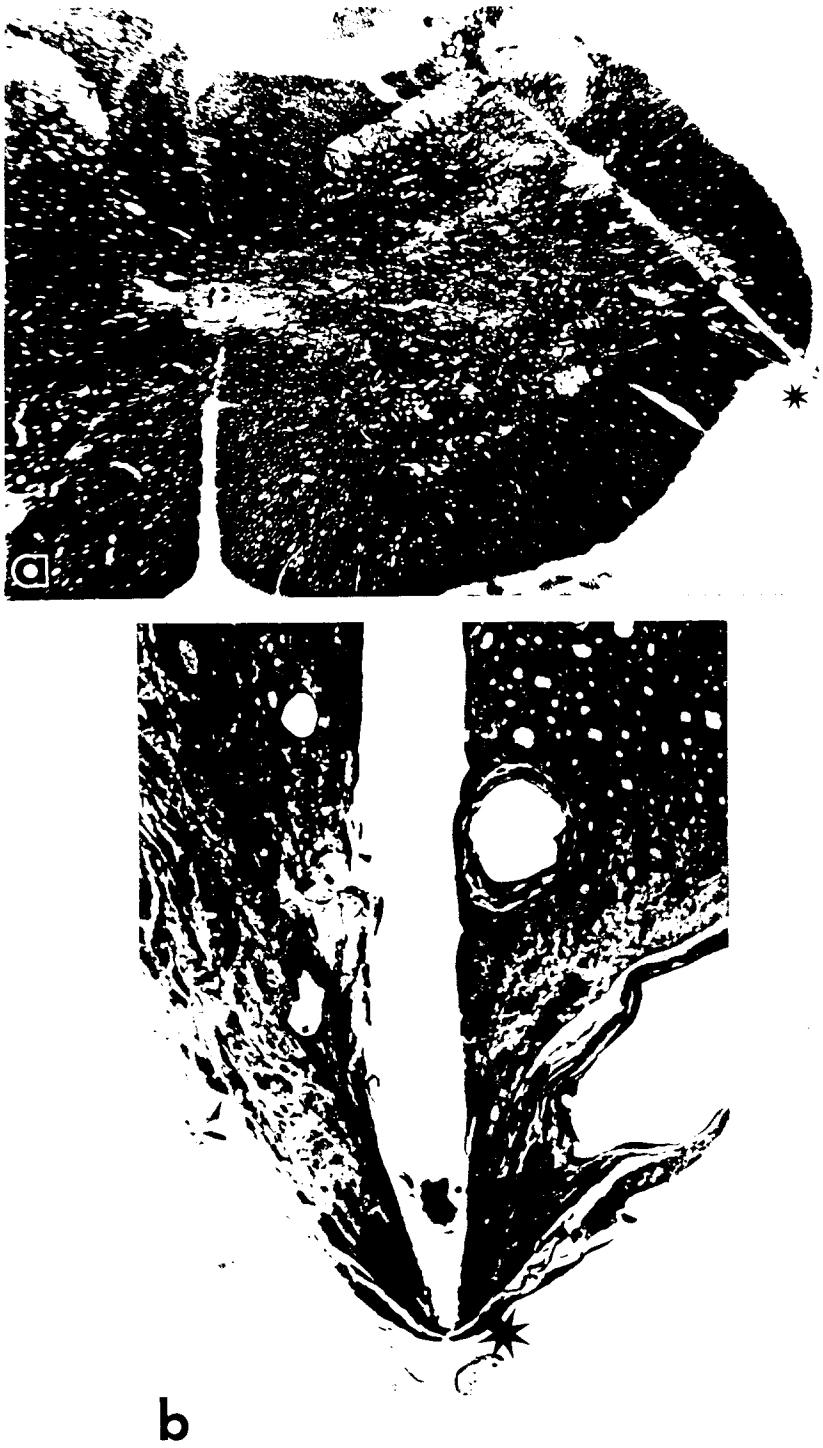

Fig. 8a,b. SP-82, both unpulsed electrodes # 4 and #5 traversed the entire hemisphere, the tips of which were observed within the ventral pia (*). Electrode #4 and its tip are shown in a and b. Bars: a = 500μm; b = 25μm.